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STUDIES ON CHROMOSOME ABERRATIONS OF LEUKOCYTES IN HUMANS EXPOSED TO RADIATION FOR MEDICAL PURPOSES

Final Report

by

C. BIAGINI *, P. BRANCADORO ** and A. SICILIANO **

* Università di Sassari

** Università di Roma

1969

Report prepared by the Istituto di Radiologia
Università di Roma - Italy

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The probability of obtaining positive cultures of lymphocytes in a group of 201 leukocyte cultures was related to erythrocyte sedimentation rate values, leukocyte counts and age.

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In different groups of patients an increase of polyploid cells was observed in 72 hours cultures of leukocytes after irradiation of a part of the body from 1.16 tetraploid per thousand diploids in 25 samples of unirradiated subjects, to 2.6 ‰ in 20 patients irradiated on a chest field with skin doses ranging from 100 to 1,200 rad, and 5.8 ‰ in a group of 5 patients after a full treatment of radiation therapy for cancer.

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KEYWORDS

CHROMOSOMES	CANCER
LEUKOCYTES	BLOOD CELLS
MEDICINE	CYTOLOGY
IRRADIATION	GENETICS
THERAPY	IN VITRO

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STUDIES ON CHROMOSOME ABERRATIONS OF LEUKOCYTES IN HUMANS EXPOSED TO RADIATION FOR MEDICAL PURPOSES*)

1. INTRODUCTION

The object of the present research is an attempt in order to obtain quantitative data on chromosome aberrations of leukocytes, induced in humans by irradiation of a part of the body carried out for therapeutical purposes. In order to find an appropriate term of reference for values of the absorbed dose, we considered not only the dose to the skin but also the integral dose and the dose to the hemopoietic tissues. The problem was discussed in preceeding reports in which a method of assessing bone marrow dose was reported (Biagini et al., 1965, c).

A collateral purpose of the research is the collection of data on possible effects on chromosomes of the radiation doses due to X-rays diagnostic exposure.

The main limitation of the study is represented by the doubt that some effects observed on a limitate number of cells grown in the culture of a blood sample are fully representative of the effects of radiation on the whole cell population after a partial exposure. That appears more relevant when only a small field of the surface of the body is exposed to radiation. However data collected after

*) Manuscript received on 7 January, 1969.

a single irradiation in various subjects could afford some useful information;the possible significance of these data will be discussed.

2. MATERIAL AND METHODS

2.1. Blood sampling

Since the patients to be submitted to radiation for therapeutical purposes are bearing cancer,avoidance of a possible influence on chromosomes of physiological conditions or of other therapeutical means than radiation (as drugs or blood transfusions) appears to reduce availability of suitable subjects. Moreover,since data obtained on subjects submitted to a full treatment with fractionated doses of irradiation are considered as giving no sufficient indications of radiation quantitative effects,another limitation is represented by the difficulties of selecting suitable patients for single irradiation.

On the whole a group of 130 subjects were submitted to blood drawing for chromosome analysis. As control subjects, 39 patients bearing malignant tumours,aging from 34 to 70,were examined before being submitted to irradiation for therapy. 37 patients were studied 24-72 hours after a single exposure,with skin doses of gamma rays

from cobalt-60 ranging from 10 to 1200 rad;29 of these were irradiated on the chest (field 10x10 cm);8 on the anterior surface of the thigh (field 15x5). 13 patients were examined during and immediately after a full treatment of radiation therapy for cancer. or after a period of 6 months - 4 years.

2.2. Technique of sampling and cytogenetic analysis

Chromosome analysis was made on cultured leukocytes prepared by using the method of Moorhead et al.(1960). 10 ml of heparinized blood was mixed with 0.20-0.30 ml of Phytohaemagglutinin;the blood was left standing at 5°C for 30 min,then centrifuged 5 min at 250 rpm. 2 ml of plasma are removed and placed in a glass container,by adding 8 ml of culture medium (TC 199 Difco),then incubated at 37°C for 70-72 hours. 2 ml of colchicine were added at a final concentration of 10^{-7} M for 2-3 hours;cells were fixed in a 3:1 mixture of methanol and glacial acetic acid and stained with Giemsa solution.

As a rule,100 cells randomly chosen were counted in every sample under the microscope (1000 enlargements);10-20 mitoses per sample were photographed and enlargements of the photographs were used to construct karyotypes;3000 cells (when possible) were counted in order to evaluate the proportion of polyploid cells. Some examples of observed abnormalities are shown in Figures 4-9.

2.3. Irradiation technique and dosimetry

The exposure of the patients was carried out with gamma rays from a cobalt-60 unit for teletherapy (FSD 55,5 cm;dose rate 32 rad/min). Dosimetry was performed with a Baldwin Ionex ionizing meter,using a 0.6 ml chamber,and a R/rad ratio of 0.975. Integral doses were calculated following the method of Scarpa (1960).

3. RESULTS AND DISCUSSION

3.1. Control subjects

Control data were obtained on 39 patients bearing malignant tumours. But of seven subjects,the patients have been submitted to one or more radiological examinations at different times before the blood sampling. The amount of exposure was recorded when possible in order to try to find a possible influence of the radiation burden due to diagnostic examination on the chromosomal picture.

3000 cells were scored in 34 samples,and we found 28 isochromatid breaks,19 fragments and 2 asymmetrical translocations (1 dicentric and 1 tricentric in the same cell). In addition to these aberrations a number of chromatid breaks was found,giving a percentage of 2.2. No chromatid interchanges were observed. Since the last two types of aberrations are commonly referred to culture effect,

they will be no further considered. On the other hand also isochromatid breaks can be attributed at least in part to the effects of manipulation (Ishihara and Kumatori,1967).

Referring to acentric fragments,in the counts of 100 cells per sample we found 0 or 1 per subject. An excess of fragments was observed in 2 patients (3 fragments),and in another subject we found a cell with a fragment and a cell with 2 asymmetrical translocations. The frequency of chromosome aberrations we found (0.7 per cent) in the small population of cancer bearing subjects is consistent with percentages observed by other authors (Court Brown et al.,1965; Court Brown et al.,1967).

The possibility that a fraction of the observed aberrations could be referred to roentgen rays exposure due to diagnostic examinations was taken into special consideration. Some data were recently referred by Schmickel (1967) on the irradiation of human leukocytes in vitro;the author used exposures varying from 6 to 48 R,corresponding to the amount of doses absorbed by patients after repeated X-ray examination,and found a significant increase of chromosomal aberrations also after lower levels of exposure. Some other information about in vivo irradiation is available in the literature (Stewart and Sanderson,1961;Conen et al.,1963;Bloom and Tjio,1964;Reisman et al.,1967). These data are dealing mainly with

X-ray examination carried out in infants;no significant results were obtained,although occasionally some aberrations as fragments or dicentrics can be found in some subjects after exposure. Also in our material no definite conclusions are reached. Apart from difficulties arising from the evaluation of absorbed doses following examinations carried out in different hospitals,the absolute number of aberrations we found in control subjects was rather small,and such as to induce serious limitations to an attempt of quantitative evaluations of the dose effect. In Tab. I are reported data obtained from 32 control subjects in which information about X-ray examinations was reasonably certain. We can notice that in seven cases without any preceeding exposure the percentage of acentric fragments was slightly inferior to the remaining subjects. On the other hand three cases which showed an excess of abnormalities did not receive a higher radiation burden compared to subjects without any chromosome aberration.

The frequency of polyploid cells was also examined,by counting 44,730 cells of 25 samples from control subjects. The average proportion of tetraploid cells resulted 1.16 per thousand diploid cells. By analysing single cases the number of tetraploid cells varied from 0 on 3000 cells to the maximum value of 2.68 per thousand (3 tetraploid on 1120 diploid cells). Only 3 subjects showed percentages higher than 2 per thousand.

3.2. Effects of the patient's conditions on cell growth

Since the present research was carried out in cancer bearing subjects, the possible influence of the conditions of the patients on the results of the chromosome analysis was considered. Before irradiation no significant differences in the chromosome aberration frequency were observed among our patients and the literature data (Court Brown et al., 1965; Court Brown et al., 1967), when the age distribution is taken into account. However Buckton et al. (1962) did not find any difference among healthy subjects and cancer patients as far as percentages of aneuploid cells are concerned. Moreover average values of tetraploid cells we have observed are consistent with those found by other authors (Ohnuki et al., 1961; Ishihara and Kumatori, 1966). The frequency of polyploid cells with diplochromosomes (endoreduplication) was negligible, in contrast to other data referred by Friedman et al. (1964) in patients with malignant tumours.

After irradiation the frequency of chromosomal aberrations in cancer bearing patients could be affected by immunological factors; on the basis of results obtained by Nowell (1967) in tubercolin-stimulated lymphocytes this possibility should be considered; however we believe this is not the case for patients examined within a short time after radiation exposure.

The more relevant fact we observed is that the patient's

conditions critically affect the growth of cultured lymphocytes. In our laboratory a high percentage of successful cultures was obtained when blood of healthy subjects was used; by using the blood of patients affected by cancer and in severe conditions the proportion of positive cultures was significantly lower. In order to look for a possible correlation between the probability of obtaining a normal growth and the conditions of the subject, data on the age, on leukocyte counts and on erythrocyte sedimentation rate (ESR) were collected; the figures were divided in classes and compared to percentage of positive cultures. For this purpose 201 leukocyte cultures of peripheral blood were considered, 165 of which obtained from tumour bearing subjects, 22 from patients affected by non malignant conditions, and 14 apparently healthy. As unsuccessful culture we classified any case in which the cell culture was regarded as unreliable owing to the following reasons: (a) small, pyknotic and agglutinated lymphocytes and absence of mitoses; (b) the same, with very rare mitoses; (c) loss of cells on account of degenerative processes or appearance of massive plasma coagulation in the culture. In healthy subjects the percentage of positive cultures resulted 100 per cent. Instead in the patients the percentage of successful cultures appeared to decrease with increasing ESR: these data are plotted in Fig. 1. No definite influence of leukocyte counts as well as of age

was evident.

It is common knowledge that ESR changes in patients affected by malignant tumours are connected with quantitative changes of albumins and globulins. However the reason of the phenomenon we observed remains unknown, since no information was available on the possible influence of the values of total proteinemia and protein fractions on the cultures. In some instances we observed strong pH changes (in air cultures),and in patients affected by lung cancer with very high ESR values we observed the appearance of a plasma clot about one hour after the preparation of the culture.

Trubowitz et al. (1966) found failure of cell growth in cultures of lymphocytes drawn from patients affected by Hodgkin disease; they attributed the fact to a growth-inhibiting factor in the serum of the patients. On the basis of our data we cannot draw a definite conclusion,and only the following observations are possible: (a) failure of cell growth appeared independent on the type of tumour; (b) some samples collected from subjects affected by high ESR values developed a normal growth of the cultures; (c) also in three cases of subjects with high ESR values owing to inflammatory conditions the blood cultures were unsuccessful.

3.3. Chromosome aberrations against radiation dose

Some data were discussed in preceeding reports (Biagini et al., 1965,a;Biagini et al.,1967),from which an increase of frequency of chromosome aberrations with increasing radiation dose was evident,with a high degree of variability. In the present report data on fragments are not considered,and only data on dicentrics and rings,other than on the number of the breaks per abnormal cells,are quoted.

The evaluation of the ratio of "breaks per abnormal cell" is based on the analysis of cases of which the photographs of every abnormal cell and enlargements for construction of kariotypes were available. For this evaluation two breaks per translocation and one break per deletion were considered. Double equal fragments were attributed to one break,taking into account when possible the position of the cell in the first or in the second division.

Data collected on 22 patients 24-72 hours after a single dose of gamma rays on a limitate field of the body are plotted in Fig. 2. Radiation doses ranged from 25 to 1000 rad to the skin;in the graph they are expressed as integral dose values,subdivided in classes with modulus of 0.2 Mgrad. In more detail,the first class comprises values of integral doses varying from 0 to 0.20 Mgrad;in the graph the central value of the class is indicated as 0.1 Mgrad;the second class comprises integral doses varying from 0.21 to 0.40 Mgrad, with central value of the class 0.3,and so on.

When radiation increases from 0.10 to 1.90 Mgrad the value of the "breaks per abnormal cell" ratio increases from 1.0 to 2.3.

In the same group of patients the percentage of dicentrics and rings against dose, expressed by central values of the classes of integral dose in Mgrad, is plotted in Fig. 3. The proportion of these aberrations is given by

$$y = \underline{c} + \underline{a} \times D^2$$

by substituting numerical values

$$y = 0.216 + 0.379 \times D^2$$

when D is expressed by Mgrad. By using g rad as dose unit, and by substituting for c the best estimate given by measurement in unirradiated subjects, the proportion of dicentrics and rings per cell becomes

$$y = 0.001 + 0.379 \times 10^{-14} D^2$$

The last form can be suitably utilized in order to compare data obtained with a field of 100-150 square cm with those resulting by the use of larger fields. We can suppose that in the irradiation of a part of the body, when fields of comparable sizes are used, the integral dose appears as an appropriate term of reference in order to compare effects on different parts of the body; but when the surfaces of the body exposed to irradiation show large differences, also when integral dose values are taken into account, an increase of effect with increasing irradiation fields is expected.

3.4. Chromosome aberrations after a full treatment for radiation therapy.

The analysis was performed in order to find further data on the increase of chromosome aberrations after higher dose levels than those reached after a single exposure. 7 samples were examined during or after a full treatment for radiotherapy, with skin total doses ranging from 1000 to 8000 rad; in any case blood drawing was carried out 24 hours after the last exposure. The results have shown a greater number of aberrations, but at the same time a further increase of the individual variability was evident. On the basis of this observation and owing to the difficulties of defining adequate terms of reference to compare results obtained in patients submitted to irradiation with different technical procedures, no further attempt was made to include these results in the dose effect curves.

Data on patients studied from 6 months to 4 years after a full treatment were discussed in preceeding reports (Brancadoro and Siciliano,1965;Biagini et al.,1967).

3.5. Data on polyploid cells

Data collected after in vitro irradiation of human leukocytes have shown an increase of tetraploid cells after doses of 400 rad of gamma rays (Ohnuki et al.,1961) or after 250-400 R of X-rays

(Bell and Baker,1965). More information is available on experimental material irradiated in vitro (Greeblatt,1961;Yu and Sinclair, 1964;Rondanelli et al.,1963). After in vivo exposure some data suggest a possible increase of polyploid cells in humans,without a definite quantitative pattern. An increase of endoreduplications was observed by Friedman et al. (1964) after total body irradiation and an increased amount of polyploid erythroblasts of bone marrow was noticed by Nemec and Polak (1964) in 8 subjects treated with therapeutical doses of radioactive iodine. More recently Ishihara and Kumatori (1966) found a proportion of 2.6 polyploids per thousand diploid cells in cultures of peripheral blood of 6 Thorotrast patients,and a proportion of 3.0 per thousand in 23 Bikini fishers. In a group of control subjects the ratio was 0.83 per thousand. An increase of polyploid cells was observed in leukocytes after in vitro exposures from 50 to 350 R of gamma rays by the same authors.

From data surveyed in the present report it would seem that an increase of polyploid cells roughly proportional to the dose appears in the 72 hours cultures of leukocytes after in vivo irradiation of a part of the body. A first comparison between the blood cultures of unirradiated subjects and those of exposed patients shows a significant increase of tetraploid cells per thousand diploids The relative proportions are 1.16 per thousand (on 44,730 counted

cells from 25 samples of unirradiated subjects) and of 2.04 per thousand (on 60,742 cells from 40 samples af irradiated patients); $\chi^2 = 6.175; P < 0.05$. In the last group patients exposed to low levels of skin doses are included probably not contributing to the observed increase of polyploids. When single groups of patients are considered, the degree of significance is higher. In a group of 20 patients, submitted to gamma irradiation on a chest field of 10x10 cm, data collected 24-72 hours after skin doses ranging from 100 to 1200 rad given a proportion of 2.6 tetraploid cells per thousand ($\chi^2 = 20,950$; $P < 0.001$). A rate of 5.8 per thousand was reached in group of 5 samples drawn from patients during or after a full treatment of radiation therapy for cancer ($\chi^2 = 30.970; P < 0.001$).

In our material we observed a high degree of variability in the response of the increase of polyploid cells against radiation dose. Furthermore some other restrictions tend to limit the significance of the increase of polyploid cells as a possible quantitative expression of radiation effects in partially exposed humans. These limitations are: (a) Spontaneous incidence of polyploid cells in cultured lymphocytes; as regards our patients the average number resulted about 1 per 1000; nevertheless in a few subjects (3 out 25) we found proportions of polyploid cells significantly higher, and

of the same order of magnitude of those of subjects exposed to smaller dose levels. (b) Possible differences in the individual radiation response. Our data are not giving clear information on this point,but such possibility was pointed out by Heddle et al. (1967) for in vitro irradiation of human lymphocytes. (c) The appearance of polyploid cells could be related to other biological mechanisms than those described by Levan and Hauschka (1953) and by Hsu and Moorhead (1956). Ishihara and Kumatori (1966) suggested that polyploid cells might be originated from affected cells with abnormal chromosomes such as dicentrics. (d) Possible dependence of polyploid rate on handling procedures during the preparation of the cultures;it is known that polyploidy can be induced on in vitro cells by variations of temperature (Ostertag,1963;Cerny et al., 1967). (e) A large number of cells must be examined in order to evaluate the proportion of polyploid cells;this restriction could be reduced by the use of computing machines.

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Tab. 1 - Chromosome aberrations in control patients classified on the basis of the X-rays diagnostic burden

X-rays diagnostic burden	Number of patients	Number of counted cells	Isochromatid breaks	Fragments	Dicentrics
0	7	622	8	2	-
+	9	868	6	6	-
+++	16	1310	13	10	2
Total	32	2800	27	18	2
Percentages			0.96	0.64	0.07

C A P T I O N S O F F I G U R E S

Fig. 1 - Probability of obtaining positive cultures of human lymphocytes against erithrocyte sedimentation ratio (ESR).

Fig 2 - "Breaks per abnormal cell" ratio against radiation dose expressed by integral dose values and subdivided in classes.Modulus 0.2 Mgrad.

Fig. 3 - Dicentrics and rings against radiation dose expressed by integral dose values and subdivided in classes.Modulus 0.2 Mgrad.

Fig. 4 - Chromatid break in a 16-18 chromosome.

Fig. 5 - Metaphase with unstable aberrations.

Fig. 6 - A dicentric and two pairs of fragments.

Fig. 7 - Abnormal chromosome due to symmetrical translocation.

Fig. 8 - A tetraploid cell with 92 chromosomes randomly distributed in the metaphasic plate.

Fig. 9 - Tetraploid cell with diplochromosomes (endoreduplication) .

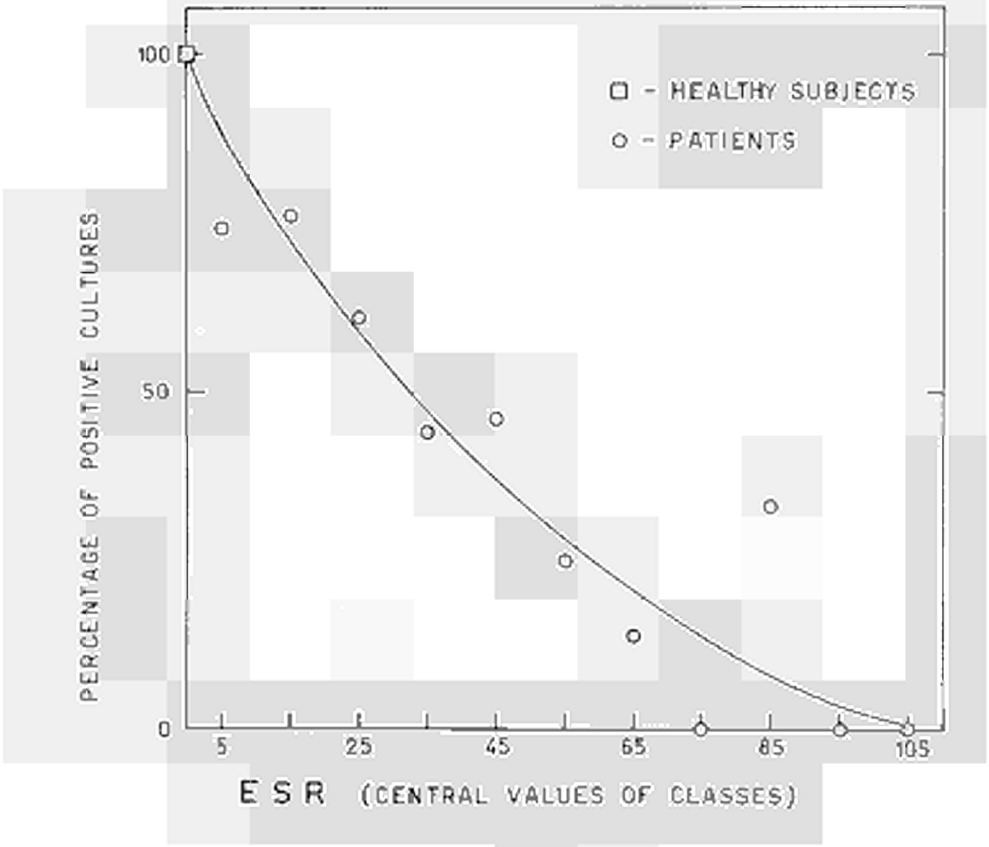
100
50
0
□ - HEALTHY SUBJECTS
○ - PATIENTS
PERCENTAGE OF POSITIVE CULTURES
5
25
45
65
85
105
E S R (CENTRAL VALUES OF CLASSES)

FIG. 1

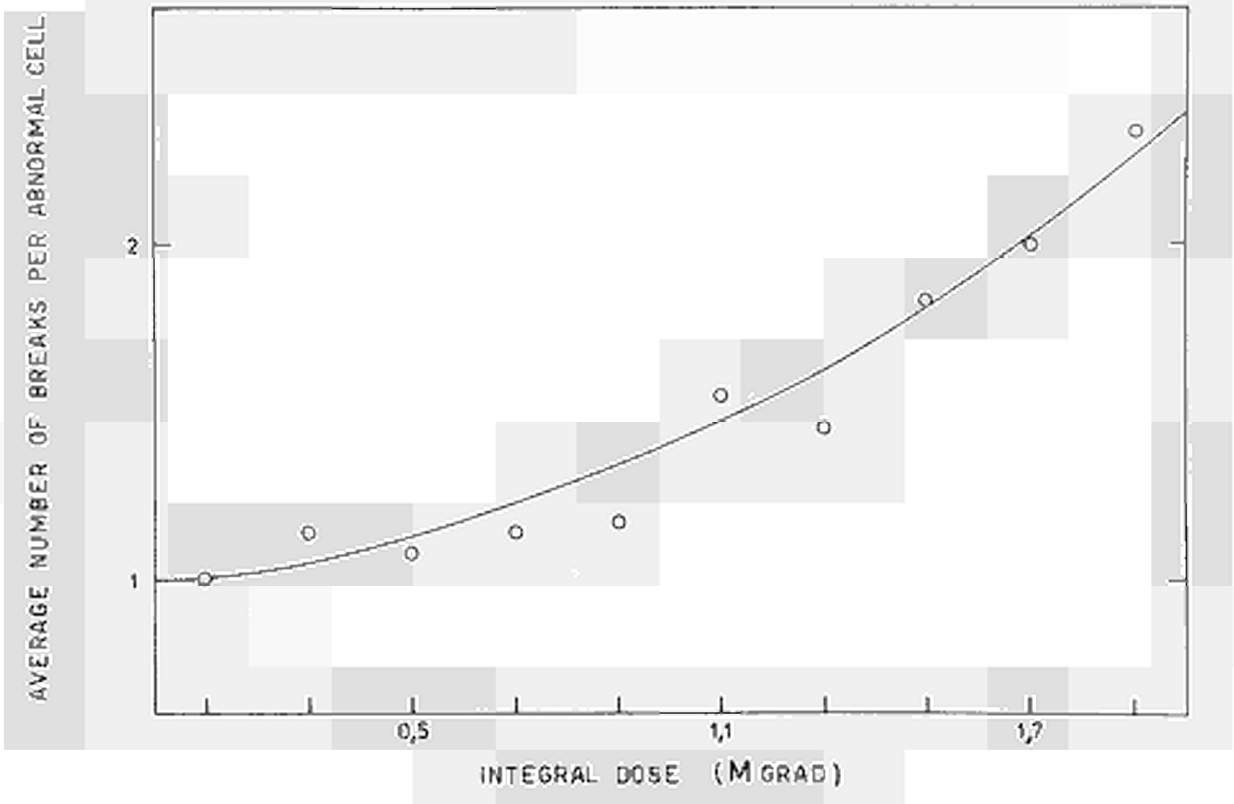
AVERAGE NUMBER OF BREAKS PER ABNORMAL CELL
2
1
0,5
1,1
1,7
INTEGRAL DOSE (M GRAD)

FIG. 2

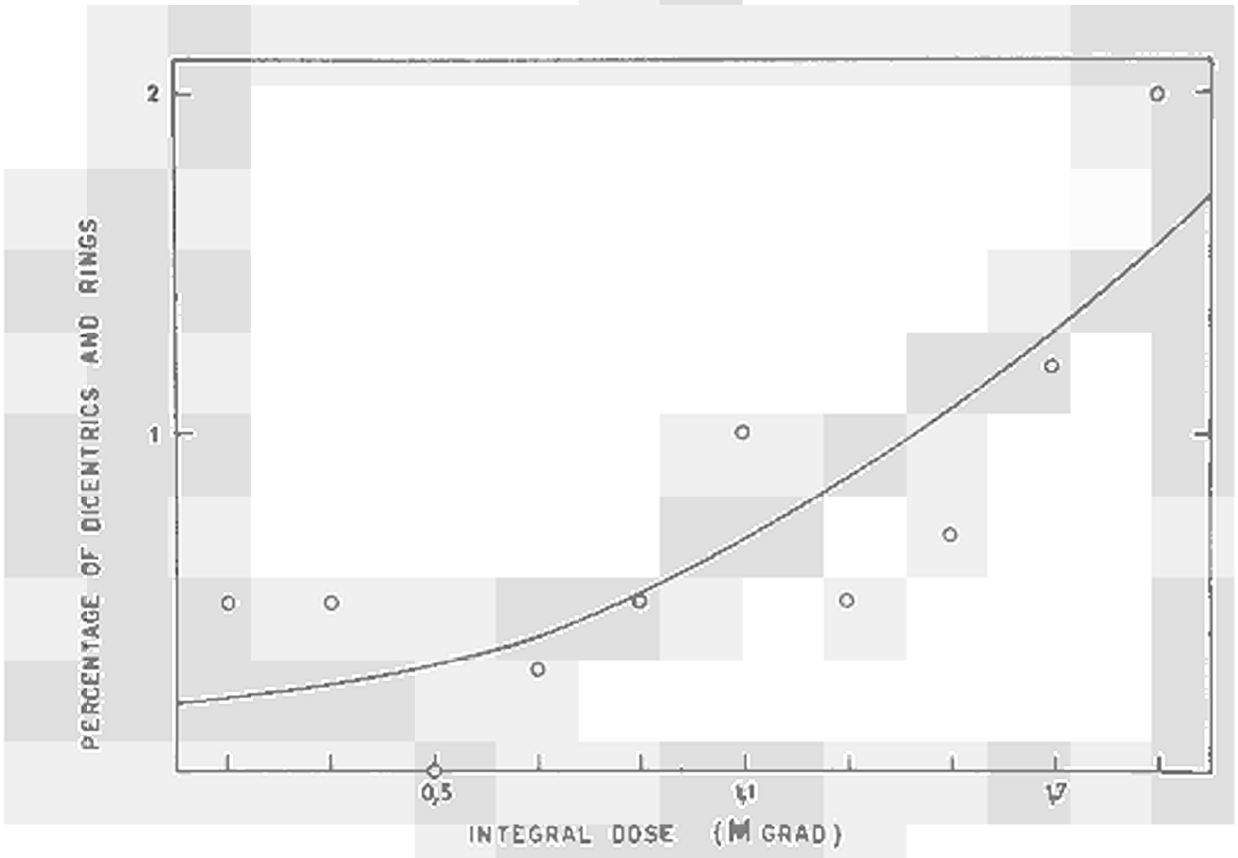
PERCENTAGE OF DICENTRICS AND RINGS
2
1
0,5
INTEGRAL DOSE (M GRAD)

FIG. 3

FIG. 4

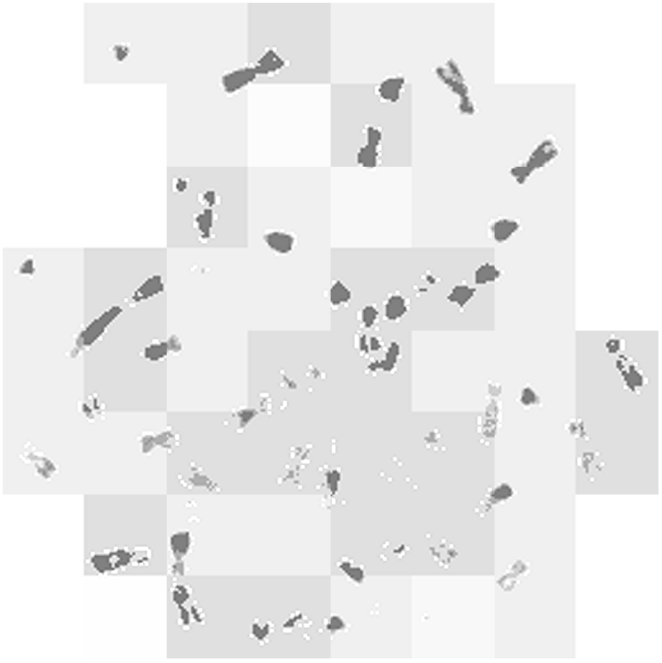

FIG. 5

FIG. 6

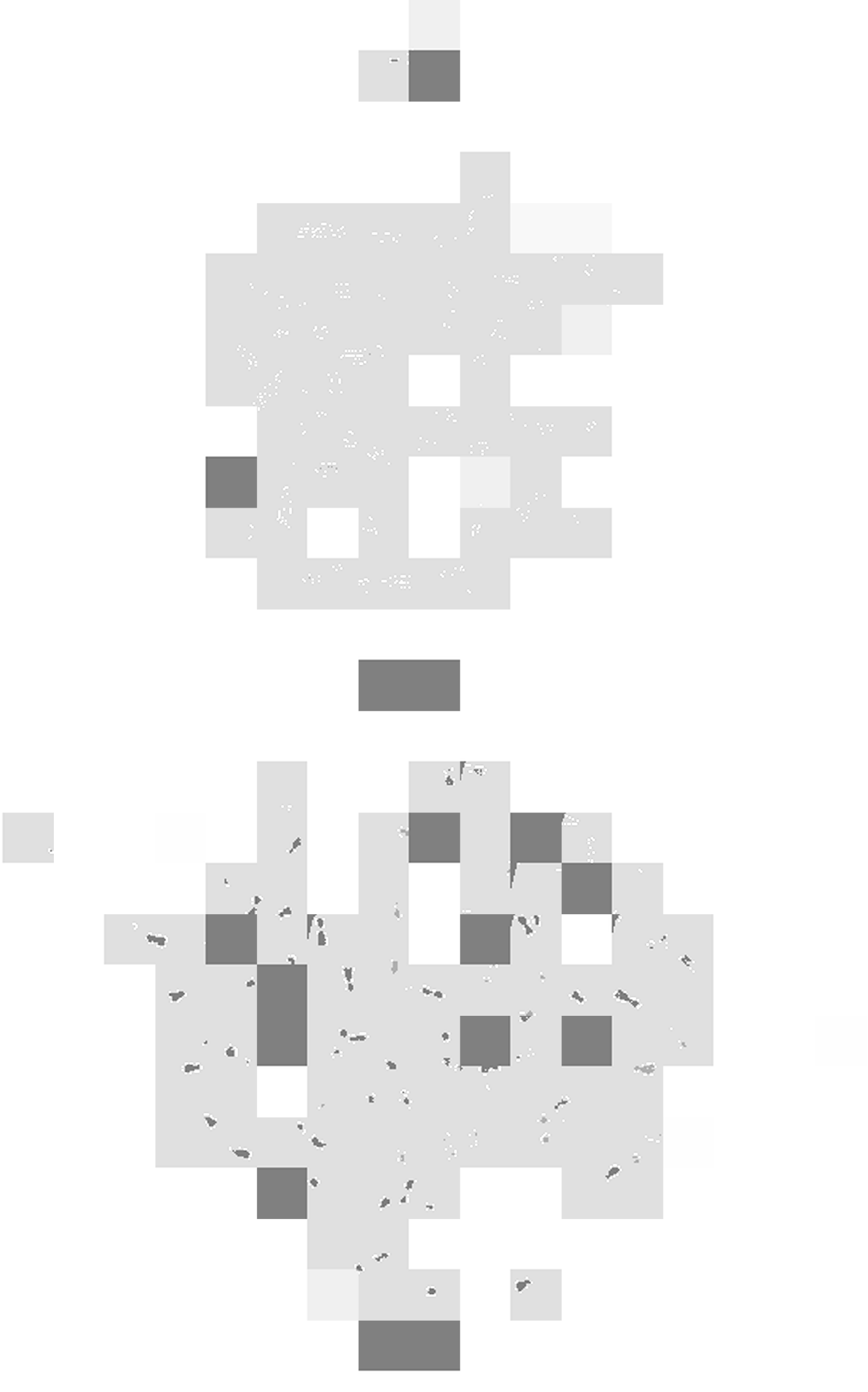

FIG. 9

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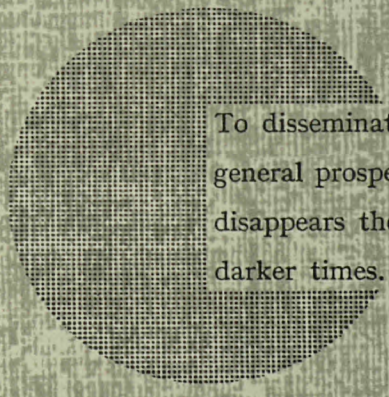

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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